

SUPPORT FOR THE AMENDMENTS

Support for the amendment to claims 7 and 9 is found on page 10, lines 3-8 of the specification. Support for the amendment to claim 12 is found in claim 1 as originally presented. Support for claims 13 and 14 is found on page 13, lines 1-8 of the specification. No new matter would be added to this application by entry of this amendment.

Upon entry of this amendment, claims 7-9 and 12-14 will now be active in this application.

REQUEST FOR RECONSIDERATION

The claimed invention is directed to a matrix for cell culture, an insect cell culture vessel coated with same and a process for preparing an insect cell culture line using a coated insect cell culture vessel.

Insect cell culturing typically comprises an initial primary culture followed by a subculture stage. During the primary culture stage, cells crawl out from a piece of fixed tissue in a process known as "migration." Efficient migration is beneficial to establishing a cell line. Coatings of extra cellular matrix on flask surfaces have heretofore provided levels of cell adhesion which could be improved. Accordingly, there is a need for an extra cellular matrix with a high cell adhesion ability for good cell culturing.

The claimed invention addresses this problem by providing a matrix for cell culture comprising a silkworm-derived water-soluble chitin having a degree of deacetylation of 45-55% obtained by treatment with concentrated alkali. Applicants have discovered that such a silkworm-derived water-soluble chitin may perform as a good matrix for insect cell culture. A matrix for cell culture comprising silk worm water-soluble chitin as claimed is nowhere disclosed or suggested in the cited prior art of record.

The rejections of claims 4-7 under 35 U.S.C. §102(b) and as obvious under 35 U.S.C. §103(a) over JP 4-6166635A, JP 4-5000954A, JP 4-4138169A and JP 4-4293481A are respectfully traversed.

A matrix for cell culture comprising a silkworm-derived water-soluble chitin is nowhere disclosed or suggested in the cited prior art of record.

JP '635A describes chitinous material used as an immunoadjuvant for promoting an immunizing affect of an antigen infused into an organism. While the immunoadjuvant may take the form of chitin, deacetylated chitin, water-insoluble chitin, slightly water-soluble chitin and a water-soluble chitin, the material is finely granulated so as to be suspended in water in use and used (see Abstract). A granulated chitin is not in a form of a "matrix for cell culture." As such, the reference fails to describe a matrix for cell culture comprising a silkworm-derived water-soluble chitin having a degree of deacetylation of 45-55%.

In contrast, the claimed invention is directed to a matrix for cell culture comprising a silkworm-derived water-soluble chitin having a degree of deacetylation of 45-55% obtained by treatment of silkworm derived chitin with concentrated alkali. Applicants note the claims have been amended to recite the source of the chitin as silkworm, the degree of deacetylation is 45-55% and that such water-soluble chitin is prepared by treatment with concentrated alkali. As the cited reference fails to disclose or suggest none of 1) silkworm as a source of chitin; 2) a degree of deacetylation of 45-55%; or 3) that the chitin is in the form of a matrix for cell culture, the claimed invention is clearly neither anticipated nor made obvious from this reference and accordingly withdrawal of the rejections under 35 U.S.C. §102(b) and 35 U.S.C. §103(a) is respectfully requested.

JP '954A describes using deacetylated chitin for the treatment of collagen diseases. There is no explicit disclosure in the translated abstract of the physical form of the chitin, however, as a therapeutic treatment it is not likely to be in the form of a matrix for cell

culture. The reference nowhere discloses or suggests 1) silkworm as the source of chitin; 2) a degree of deacetylation of 45-55% or 3) that the chitin is in the form of a matrix suitable for cell culture. In the absence of these claim limitations, the claimed invention is clearly neither anticipated nor rendered obvious by this reference.

Applicants note that chitin is generically a polymer of glucosamine. However chitin derived from an insect such as a silkworm as well as some crustacean such as a crab and shrimp has an α -structure which is different from a cephalopod derived chitin such as a squid, which has a β -structure. Chitin having an α -structure has large porous spaces typically containing diphenol compounds in the pores. Chitin having a β -structure has small porous spaces and typically comprises calcium carbonate in the pores. The attached references, with partial translations, by *Haga et al.*, J. Seric. Sci. Jpn. (Exhibit B), National Institute of Sericultural and Entomological Science, COE (Exhibit C) and from a National Institute of Sericulture and Entomological Science COE News Letter (Exhibit D) make clear the structural difference between α -chitin and β -chitin structures.

JP '169 merely describes a medical device to cure an intractable trauma comprising a composite body of chitin or a chitin derivative with a reinforcement suitable chitins include chitins from natural materials, deacetylated chitins, etherified chitins, carboxymethylated chitins, hydroxyethylated chitins, acetylated chitins and sulfonated chitins. There is no disclosure in the reference of 1) a silkworm source of chitin; 2) the specific degree of deacetylation of 45-55% or 3) that the chitin is in the form of a matrix for cell culture. Accordingly, the claimed invention is neither anticipated nor made obvious from this reference.

JP '481A describes a petri dish for culturing a cell having a differentiation character such as a hepatic cell which contains a coating of chitin, chitisan, cellulose or derivatives. The reference only discloses a chitin or derivative thereof derived from squid as evidenced by

the partial translation of claim 1 as well as example 1 of JP '481A (Exhibit A). As previously discussed, chitin obtained from squid is of the β -structure and accordingly does not suggest the silkworm derived chitin containing the α -structure as claimed.

An advantage of using silkworm derived chitin is as follows:

When primary cell culture of insect cells is carried out, a cut piece of insect tissue is placed on the matrix for cell culture. Since silkworm derived chitin has large pore spaces, the matrix becomes spongy and a portion of the piece of cut tissue can be embedded in the matrix to be fixed. Then, cells crawl out from the cut piece of the fixed tissue. The crawling out of cells is called "migration." It is helpful to have efficient migration to establish a cell line (see attachment with partial translation by *Umetani* Agriculture, Forestry and Fisheries Technical Information Society (Exhibit E)). However, in order for cells to migrate efficiently, it is helpful that the tissue piece is firmly fixed. When chitin derived from a silkworm is used, a portion of the tissue of the insect is embedded into the pore spaces of the chitin and firm anchorage is established. Therefore, a tissue piece is firmly fixed to the chitin and cells can easily migrate from the tissue piece. Consequently a cell line can be established efficiently. Moreover, as a tissue piece used for primary cell culture is composed of epithelial cells, both the matrix and the tissue piece are derived from epithelium, such that the tissue piece can be closely fixed to the cellular matrix.

As the cited reference fails to disclose the claim limitations of 1) chitin from silkworm or 2) the specific deacetylation degree of 45-55% the claimed invention is clearly neither anticipated nor made obvious from this reference and accordingly withdrawal of the rejection under 35 U.S.C. §102(b) and 35 U.S.C. §103(a) are respectfully requested.

The rejections of claims 4-12 under 35 U.S.C. §112, second paragraph have been obviated by appropriate amendment.

Applicants have now amended claim 7 to replace the phrase "extra cellular matrix" with the phrase --matrix for cell culture--. Applicants respectfully submit that the phrase "a matrix for cell culture comprising a silkworm-derived water-soluble chitin..." is sufficiently clear to those of ordinary skill in the art and those skilled in the art to understand the metes and bounds of the claimed invention as the chitin is in the form suitable for cell culture. Claim 11 which previously recited "a short period of time" has now been amended to remove this phrase. In view of applicants' amendments, withdrawal of these grounds of rejection is respectfully requested.

Applicants submit that this application is now in condition for allowance and early notification of such action is earnestly solicited.

Respectfully submitted,

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